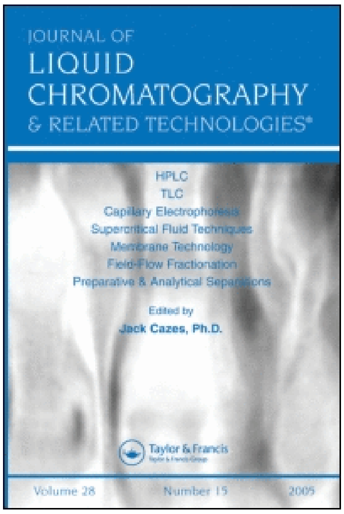

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Computational Chemical Simulation of Chromatographic Retention of Phenolic Compounds

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ABSTRACT

An *ab initio* simulation of reversed-phase liquid chromatography for phenolic compounds was developed using a molecular mechanics calculation in the CAChe™ program. The calculated molecular interaction energy values were well correlated with the log *k* values measured as molecular and ionized forms in reversed-phase liquid chromatography. Molecular interaction in liquid chromatography can be quantitatively estimated from the energy values calculated by molecular mechanics using analytes and a model phase.

Key Words: Molecular interaction; Liquid chromatography; Computational chemistry; Phenolic compounds.

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INTRODUCTION

Liquid chromatography and computational chemistry have been used for various processes in drug discovery. Liquid chromatography is a purification technique and a measurement method of the physico-chemical properties of molecules. Quantitative analysis of the retention mechanisms in liquid chromatography, using computational chemistry, should improve the precision of molecular properties measured by liquid chromatography. Furthermore, an *ab initio* prediction method can be developed if experimental data measured under the same conditions are available. Previously, the retention time in reversed-phase liquid chromatography was quantitatively analyzed using $\log P$ values. Retention times of phenolic compounds and aromatic acids in a given pH eluent in reversed-phase liquid chromatography were predicted from their dissociation constants, derived from their atomic partial charges and $\log P$ values calculated by a computational chemical method.[1] Their maximum retention times in the molecular form were derived from their $\log P$ values. The precision of the calculated atomic partial charges by AM1 of MOPAC$^{\mathrm{TM}}$, using the CAChe$^{\mathrm{TM}}$ program,[2] was acceptable to predict pK_a values of phenolic compounds and aromatic acids.[3]

In the *ab initio* approach, the maximum retention factor in the molecular form can be obtained from $\log P$, however the minimum retention factor in the 100% ionized form could not be predicted mathematically. That is, the retention factor at pH 7.4, a physiological condition to measure drug-protein binding affinity, could not be predicted with high precision. Therefore, the chromatographic behavior of phenolic compounds was analyzed by a computational chemical method using the CAChe$^{\mathrm{TM}}$ program. The molecular interactions between a model phase and a molecular or an ion-form phenolic compound were directly analyzed by MM2 calculation, using the CAChe$^{\mathrm{TM}}$ program, and the energy difference was related to the retention factors measured by liquid chromatography. The addition of pK_a values improved the estimation of molecular interaction in a given pH eluent.

EXPERIMENTAL

Reagents and Materials

Derivatized phenols were obtained from Wako Pure Chemical Industries (Osaka, Japan). Their properties are summarized in Table 1 with their retention factors, as measured by reversed-phase liquid chromatography. Sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate $12H_2O$

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Table 1. Molecular properties of standard compounds.

Chemicals	$V\log P$	pK_a[a]	pK_a[b]	pK_a exp	$\log k_{\max}$	$\log k_{\min}$
Benzoic acid	1.485	4.20		4.23	0.399	−0.827
Phenol	1.587	10.36	9.92	9.94	0.152	−0.785
4-Chlorophenol	2.469	9.74	9.39	9.02	0.649	−0.368
2,4-Dichlorophenol	3.151	4.47	7.87	7.58	1.034	−0.089
2,4,6-Trichlorophenol	3.761	6.65	6.35	5.86	1.276	0.497
2,3,4,6-Tetrachlorophenol	4.311	5.63	—	5.25	1.628	0.855

[a] From Ref.[9].
[b] From Ref.[10].

were purchased from Wako Pure Chemical Industries. HPLC-grade methanol was obtained from Kanto-Kagaku (Tokyo). Water used was Milli-Q grade.

Liquid Chromatograph

The liquid chromatograph was constructed with a model LC-10AD pump and a model SIL-10AXL auto-injector, a model SPD-10AV UV detector from Shimadzu (Kyoto, Japan) equipped with a model UZ-SH-MIC μflow cell from LC Packing (The Netherlands), and a model ERC-3522 degasser from ERC (Tokyo). The aluminum block column heater was made to specifications and controlled with a model 965 Temperature & Process Controller from Sakaguchi E.H. Voc Co. (Tokyo). The operation and chromatographic data analysis were performed with a CLASS-LC10 workstation from Shimadzu.

Measurement of Retention Factors of Acidic Compounds in Reversed-Phase Liquid Chromatography

A pentyl bonded silica gel column,[4] 50×2.1 mm I.D., was used for reversed-phase liquid chromatography with various pH eluents. The eluent was a 2 : 1 mixture of pH-controlled 50 mM sodium phosphate solution and methanol. The flow rate was $0.2\,\mathrm{mL\,min^{-1}}$. The column temperature was 37°C. The void volume marker was fructose.

Computational Chemical Analysis

A model butyl-bonded phase was constructed for studying the molecular interactions in reversed-phase liquid chromatography, because an analyte could not slip into the highly dense alkyl-bonded phase and the pentyl-bonded silica gel did not show silanol effect.[4] The model butyl-bonded phase consisted of 628 carbons and 216 hydrogens and 1197 bonds and 6768 connectors. The molecular weight was 7752. The molecular design is due to the capacity of a computer used. The optimized energy value was less than 0.00001 kcal mol^{-1}. The adsorption form of phenol in the butyl-phase is shown in Fig. 1. After subtraction of the individual energies of the analytes and the butyl-phase from the molecular interaction energy values, the retention factors obtained by liquid chromatography were related to their final structure (FS), hydrogen bonding (HB), electrostatic (ES), and van der Waals (VW) energy values calculated by MM2 and are listed in Table 2.

RESULTS AND DISCUSSION

The interaction between a molecular form compound and the butyl-phase was calculated to analyze the retention of molecular form analytes. That between an ionized form compound and the butyl-phase was calculated to analyze the retention of ionized analytes. The subtracted energy values (Δvalues) of the FS (optimized structure), and VW, were well correlated with the log k values of molecular and ionized form phenolic

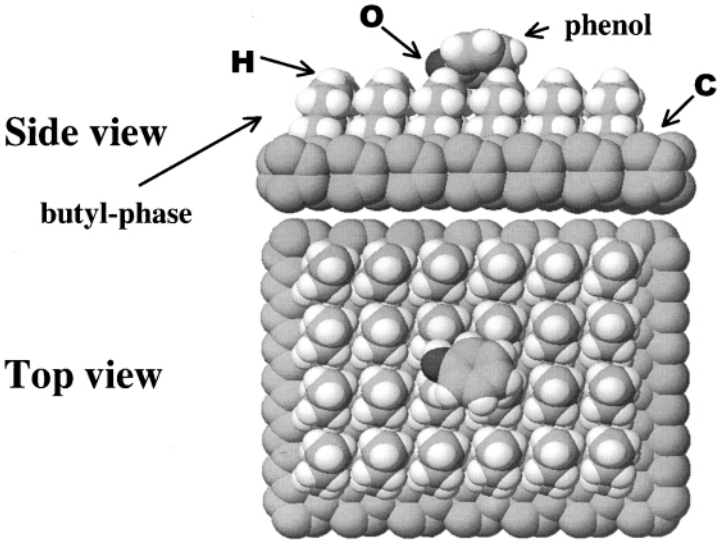

Figure 1. Adsorption of phenol on the model butyl-bonded phase.

Table 2a. Optimized individual and complex energy values of molecular form by MM2 (kcal mol^{-1}).

	Model butyl-phase with a molecular form compound							
	Individual				Complex			
Chemicals	FS	HB	ES	VW	FS	HB	ES	VW
Benzoic acid	−13.8831	−3.401	−6.632	4.829	3351.661	−3.475	−6.682	417.184
Phenol	−10.2105	−1.477	0	2.960	3356.070	−1.487	0	416.126
4-Chlorophenol	−10.0243	−1.481	−0.087	3.155	3355.213	−1.462	−0.087	415.194
2,4-Dichlorophenol	−10.4377	−1.918	−0.556	3.653	3354.364	−1.904	−0.555	415.106
2,4,6-Trichlorophenol	−12.6817	−1.980	−3.211	4.108	3350.713	−1.959	−3.207	414.148
2,3,4,6-Tetrachlorophenol	−4.5807	−1.968	2.356	6.101	3358.255	−1.967	2.353	415.411
Model butyl-phase	3373.0371	0	0	419.968				

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Table 2b. Optimized individual and complex energy values of ionic form by MM2 (kcal mol^{-1}).

	Model butyl-phase with a molecular form compound							
	Individual				Complex			
Chemicals	FS	HB	ES	VW	FS	HB	ES	VW
Benzoic acid	−2.549	0	0	4.743	3363.323	0	0	417.427
Phenol	−7.676	0	0	2.986	3358.753	0	0	416.333
4-Chlorophenol	−8.019	0	−0.618	3.182	3357.297	0	−0.618	415.302
2,4-Dichlorophenol	−7.160	0	−0.225	3.587	3357.648	0	−0.225	415.170
2,4,6-Trichlorophenol	−8.835	0	−2.362	4.055	3354.650	0	−2.369	414.269
2,3,4,6-Tetrachlorophenol	1.330	0	4.240	6.907	3364.574	0	4.241	416.950
Model butyl-phase	3373.0371	0	0	419.968				

compounds. The correlations between the substituted energy values of the FS and the $\log k$ values of molecular and ionized form phenolic compounds were obtained from the following equations:

$$\log k_{\mathrm{mol}} = 0.419 \times (\Delta\mathrm{FS\ energy\ values}) - 2.730, \quad r^2 = 0.986, n = 6,$$

$$\log k_{\mathrm{mol}} = 0.384 \times (\Delta\mathrm{VW\ energy\ values}) - 2.505, \quad r^2 = 0.991, n = 6,$$

$$\log k_{\mathrm{ion}} = 0.494 \times (\Delta\mathrm{FS\ energy\ values}) - 4.185, \quad r^2 = 0.976, n = 6,$$

$$\log k_{\mathrm{ion}} = 0.435 \times (\Delta\mathrm{VW\ energy\ values}) - 3.776, \quad r^2 = 0.981, n = 6,$$

where $\log k_{\mathrm{mol}}$ is the $\log k$ values of the molecular form, $\log k_{\mathrm{ion}}$ is the $\log k$ values of the 100% ionized form, FS and VW represent the final structure and van der Waals, respectively.

The correlation obtained for the molecular forms was used to predict the maximum retention factors of these compounds, and that for the ionized forms was used to predict the minimum retention factors of these compounds. Furthermore, these retention factors were used to predict retention factors with different pH eluents using the following equation:

$$k = \frac{k_o + k_i(K_a/[\mathrm{H}^+])}{1 + (K_a[\mathrm{H}^+])}$$

where k_o is the maximum retention factor of the unionized form of the analytes. The term k_i is the retention factor of the fully ionized compound; K_a is the dissociation constant; and $[\mathrm{H}^+]$ is the hydrogen ion concentration of the eluent. For evaluation of the above approach, the measured pK_a values were used for the calculation. Several approaches have been proposed for the prediction of pK_a values, but it is still difficult to obtain precise values.[3]

A comparison of the predicted and measured retention factors at different pH is summarized in Fig. 2 with r^2 values. This simulation method should work for the prediction of retention factors in reversed-phase liquid chromatography, however, further study is required to determine if this new approach will work for a variety of compounds.

The retention mechanisms in a graphitic carbon phase were mainly hydrophobic interactions where the VW energy values were reduced by the adsorption of a molecule in the model graphitic carbon phase. Anions were retained, but not cations, in the model graphitic carbon phase where the atomic partial charges of the model graphitic carbon phase were reduced by the adsorption. These quantitative analyses were performed by molecular mechanics (MM) and AM1 calculation of MOPAC in the CAChe™ program.[5] Furthermore, molecules including their ion-forms were adsorbed on a model alkyl-bonded phase where the VW energy values were reduced but not the HB and ES energy

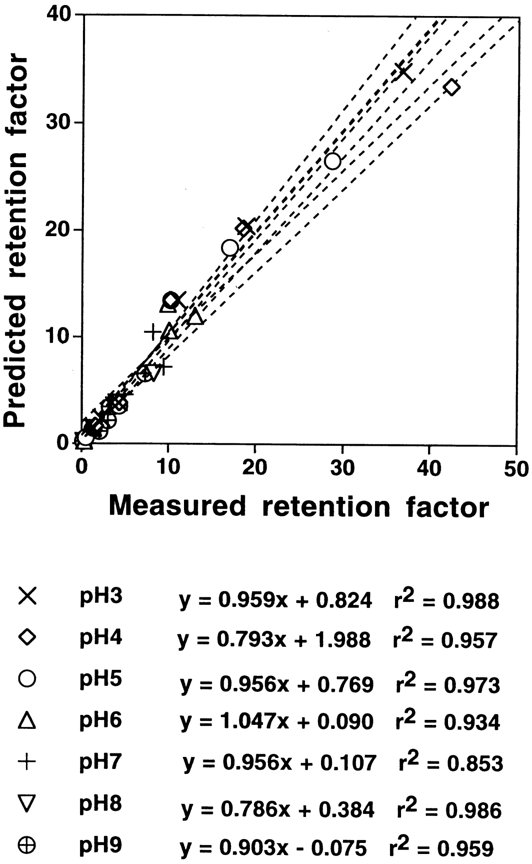

Figure 2. Relation between measured and predicted retention factors.

values, as explained in this report. Molecular form compounds were retained in a model ion-exchange phase where the VW and HB energy values were reduced, and the ionized compounds did in the phase where the ES energy values were reduced.[6]

This type of approach should demonstrate the possibility of *ab initio* estimation of albumin-acidic drug binding affinity based on the data measured previously, where the human serum albumin-drug binding affinity was measured indirectly without albumin. The predicted binding affinity was constructed from the retention factors measured by reversed-phase and ion-exchange liquid chromatography in sodium phosphate buffer, pH 7.40 at 37°C.[7,8] The addition of solvation effect, and the construction of partially ionized formed compounds should improve the precision for the quantitative analysis of retention mechanisms in liquid chromatography and, furthermore, for the prediction of protein-drug binding affinity.

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